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AGAR AND OTHER SEAWEED GUMS: A SUMMARY OF DATA ON CHEMICAL AND PHYSICAL PROPERTIES

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Just prior to World War II about 92 percent of the agar used in this country was imported from the Orient, and about 20 percent of this was used in the preparation of bacteriological culture media. During the war there were no imports, but the War Production Board was able to satisfy all essential domestic and lend-lease requirements by stock piling available supplies, and controlling withdrawals. There was also an expanded production of agar in Southern California from domestic and Mexican Gelidium seaweed. Fortunately, a really critical situation was averted and the limitation order M-96 was revoked on August 14, 1944.

During the war, staff members of the Service were assigned to investigate the possibility of expanding the production of agar, and to find gums extracted from domestic seaweeds which could be used in the replacement of agar. Surveys were conducted along the East and West Coasts to locate potential supplies of seaweeds. In California, staff members of the Service and the Scripps Institution of Oceanography cooperated in studies on the biology of seaweeds, methods of harvesting, and methods for increasing extraction yield and preparing a satisfactory product. In the East, staff members of the Service conducted experiments to determine whether gums extracted from seaweeds other than Gelidium could be made suitable for use in bacteriological culture media. Considerable work was also done to obtain data on chemical and physical properties which might be incorporated in standards of acceptability for agar, and to correlate these findings with bacteriological data.

At various times, the seaweeds listed in Table 1 have been reported as sources of agar. Of these seaweeds, those listed in Table 2 have been reported on the Atlantic Coast.

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Note: This leaflet supersedes Sep. 44, a reprint from Fishery Market News, November 1943, pages 1-5.

Table 1 - Seaweeds Reported as Sources of Agar.

Seaweed	Reported Occurrence	Remarks
Ahnfeltia plicata	Russia (Maritime Coast and White Sea)	Maritime grade best
Camphylaephora hynaeoides	Japan, China	
Endocladia muricata	California	Reported originally by Field 1/ (1921)
Eucheuma spinosum		Reported used to a considerable extent in these countries.
isiforme	Japan, China	
denticulatum		
Gelidium amansii	Japan, China, California	These seaweeds are main source of agar in Japan, China, and California.
cartilagineum	Japan, China, California, South Africa	
corneum	Japan, China, California	
australe	California	Gel similar to that from Irish moss.
pristoides	South Africa	Gum difficult to dissolve, low gel strength, opaque.
Gigartina asperifolia		These seaweeds require special treatment to obtain gum.
canaliculata	California	
serrata		
Gloiopeltis	California	
Gracilaria confervoides	Japan, China, Australia, South Africa, California	This seaweed is main source of agar in Australia and South Africa.
lichenoides	Japan, China, Australia	
Phyllophora nervosa	Russia (Black Sea)	
rubens		
Pterocladia lucida	Australia	This genus is closely related to *Gelidium*.
capillaceae	Brazil	
sp.	California	
Suhria vittata	South Africa	Gel similar to that from Irish moss.

1/ I. A. Field (1921) Sources, Preparation and Properties of Algal Gelatines. Chemical Age (NY) 29:485-486.

Table 2 - Atlantic Coast Seaweeds Reported to Contain Agar-like Gums.

Seaweed	Occurrence
Ahnfeltia plicata	Northeast coast from New Jersey to Arctic Circle. Perennial growing in rocky habitat.
Eucheuma isiforme	Florida. Occurs in some fair patches.
Gelidium corneum	One plant found in Florida. Never before reported there.
Gracilaria confervoides	Northeast coast in shallow warm and quiet bays. North Carolina coast, Beaufort region. Florida.
Gracilaria multipartita	North Carolina coast, Beaufort region.

There is some confusion as to what is meant by the term "agar." The U. S. Pharmacopoeia XII defines agar as "the dried mucilaginous substance extracted from Gelidium corneum (Hudson) Lamouroux and the other species of Gelidium (Fam. Gelidiaceae) and closely related algae (Class Rhodophyceae)." The War Production Board in its enforcement of Order M-96 defined agar as ".....any mucilaginous substance, whether dried or in other form, extracted from Gelidium corneum, Gelidium cartilagineum, Gelidium amansii, Gracilaria confervoides, Gracilaria lichenoides, Eucheuma speciosum, Eucheuma isiforme, Eucheuma denticulatum, Gigartina spinosa, Gigartina mamilosa, and from other species of the genera named above and closely related algae of the class Rhodophyceae. It is also known as 'agar agar', 'Chinese gelatin', and 'Japanese gelatin'." Neither of these definitions indicates the physical or chemical properties which are requisite in a satisfactory material for bacteriological culture media.

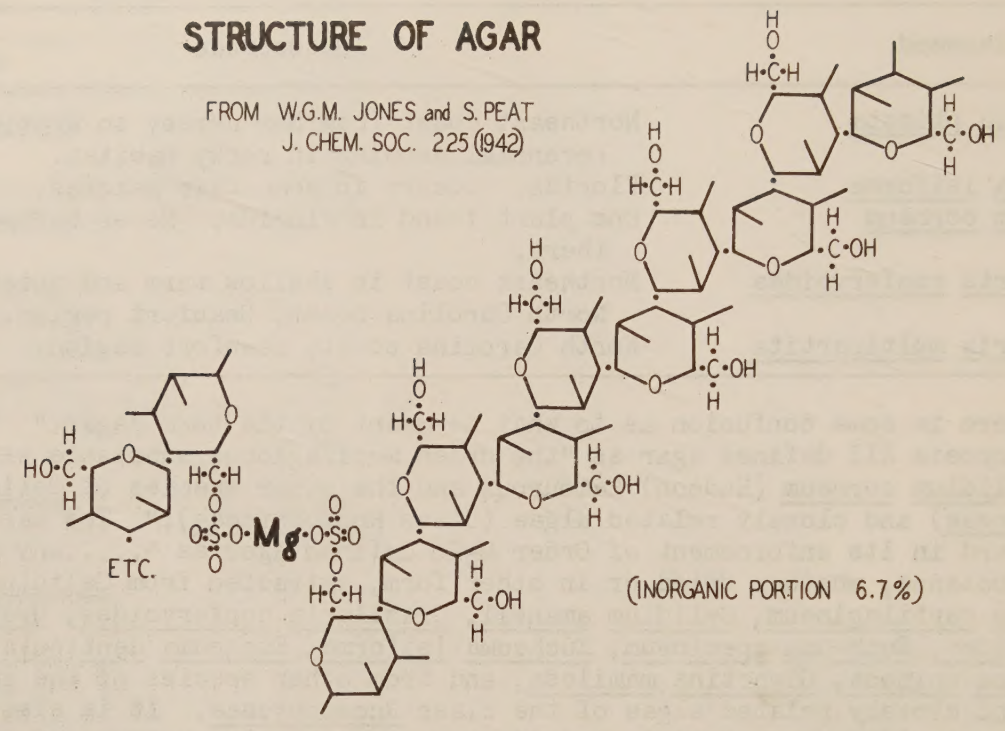
The agar of commerce is a gum common to many red algae. When the dried gum is immersed in water it will swell to many times its former volume. Agar will take up as much as ten times its own weight of water in this manner. On heating a suspension of agar in water to approximately 90° C., it will form a solution which sets to a firm gel on cooling. The unique qualities of agar are the low concentrations in which it will set to a firm gel (as low as one-half percent of good quality agar will set) and the particular temperature range in which the transition from sol to gel takes place (approximately 37-39° C.). The melting point of the gels formed is the same as the temperature of solution. This hysteresis effect may be explained by the theory that the gelation of agar is caused by a precipitation of the agar phase in the form of micelles which hold the liquid by a sponge-like mechanism. Melting of the gel requires resolution of the agar. The analogy to a sponge permits understanding of other physical phenomena of agar gels, such as syneresis.

Chemically, agar is the sulfuric acid ester of a linear galactan. A structural formula has been proposed by Jones and Peat[1] (see attached figure).

1/ Jones, W. G. M. & Peat, S. (1942) J. Chem. Soc. 161, 225

STRUCTURE OF AGAR

FROM W.G.M. JONES and S. PEAT
J. CHEM. SOC. 225 (1942)



However, the variation of characteristics between some samples, and the observation of fractions of different solubility within a sample, would lead one to expect that agar is a mixture of molecules of varying size and complexity probably built around the structure found by Jones and Peat.

The hydrolysis of the ester structure by acid and heat destroys the gelling power. This structure also indicates that there is an irreducible ash content which varies between two and three percent as found by actual determinations. The sulfuric acid ester structure requires the presence of metallic ions, and these are predominately magnesium and calcium.

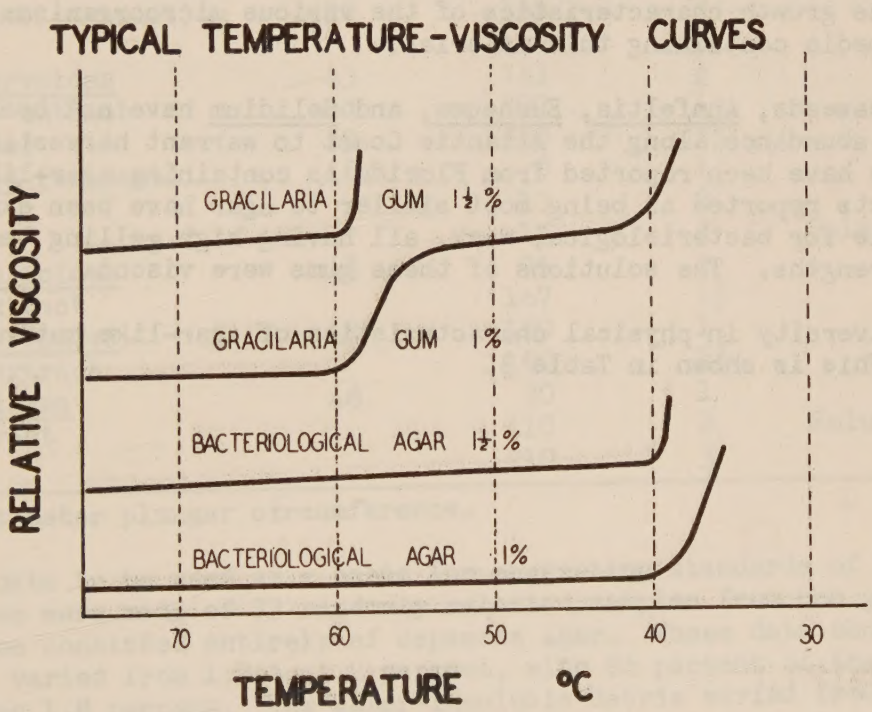
When the Fish and Wildlife Service started its investigations, there were two established seaweed-gum industries: the extraction of alginates from the kelps Macrocystis pyrifera in California and Laminaris species in Maine and the extraction of carrageenin from Irish moss (Chondrus crispus) which was gathered along the Atlantic Coast from Cape Cod northward into the Canadian maritime provinces. The literature describes Chondrus or carrageenin as the sulfuric acid ester of a galactan. The basic structure appears to be similar to that of agar. The normal ash content, however, is much greater (approximately 20 percent of the dry weight) and concentrations of five percent of the dry gum are required to form a quasi-gel. This gel is soft, tends to flow at 37° C., and is very opaque. The hot solution of the gum is extremely viscous.

Since the gathering of Irish moss was already an established industry, an attempt was made to improve the gelling properties of the gum extracted from it.

This may be done by the addition of various salts to the gum solution. However, the use of these salts is objectionable to the bacteriologist, and the gel remains opaque. After first washing out the more easily soluble fractions of the weed, the remaining more difficultly soluble portions may be extracted. These latter portions have successively greater gel strength. An extract capable of forming a fair gel in three percent concentration has been prepared, but the gel is still opaque and the warm solution, viscous. An improved extract of Irish moss having these same properties is on the market. This material can be used for various special bacteriological purposes, but should not be regarded as a substitute for agar in standard techniques. The physical differences of the material should also be kept in mind. Walker and Day[1/] have studied the use of Chondrus extract for bacteriological work and have described its values and limitations.

The presence of the seaweed Gracilaria confervoides in great abundance along the North Carolina Coast was brought to our attention by the Duke University Marine Laboratory. The properties of the gum from this seaweed were studied very carefully, since it is superficially similar to agar and has been reported as being used as a source of agar in five other regions of the world.

In the same concentrations this gum forms transparent gels of the same strength as commercial agar. However, the temperature of transition from sol to gel is higher than for normal agar. In fact, there are two transition temperatures.



1/ Walker, A. W. and Day, A. A. (1943) Food Research 8, No. 6, 435-443.

When the viscosity of agar solutions is followed as they cool, a sudden rise is noted at the transition temperature. In such studies, however, the viscosity of Gracilaria gum at the lower concentrations shows two sudden rises. These data were interpreted to mean that the gum contained at least two components, but attempts to effect a separation have been unsuccessful. Gracilaria gum gels also exhibit excessive syneresis. In 24 hours, they will exude 3 to 5 times as much liquid as similar concentrations of normal agar. This large amount of free liquid causes the gel to slip in its container and induces spreading colonies when the gum is used for bacteriological media.

Although Gracilaria gum is unsuitable for bacteriological work, it is similar enough to agar to replace it for some industrial purposes. This gum is now being produced commercially, and has been used in pharmaceuticals and foods. Some experimental amounts have also been purchased for the preparation of a lubricant used in the drawing of tungsten wire for the manufacture of incandescent electric lamps.

Gum extracts of Gracilaria confervoides from three other sources, California, South Africa, and Australia, have been examined. The gums from California and Australia were markedly similar to each other in physical properties, but differed from those of our Atlantic Coast or South Africa. They were not, however, suitable for bacteriological work because of low gel strength and high transition temperature from sol to gel. The single example tested from South Africa was different from the other three in physical characteristics and was the only one with physical characteristics suitable for bacteriological purposes. However, the growth characteristics of the various microorganisms may not be the same with media containing this material.

The seaweeds, Ahnfeltia, Eucheuma, and Gelidium have not been found in sufficient abundance along the Atlantic Coast to warrant harvesting. A number of seaweeds have been reported from Florida as containing agar-like gums. Seaweed extracts reported as being most similar to agar have been examined. None was suitable for bacteriological work, all having high gelling temperatures and low gel strengths. The solutions of these gums were viscous.

The diversity in physical characteristics of agar-like materials soon became evident. This is shown in Table 3.

Table 3 - Properties of Seaweed Extracts.

Product	Gelling Temperature °C	Breaking load grams*	Concentrations percent	Remarks
Irish moss extract		0	2	
		10	3	Solution
		75	5	viscous
Irish moss extract (prewashed weed)		165	3	Solution viscous
Irish moss extract (commercial, improved)	31	15	1	
		90	3	Solution
		300	5	viscous
Strip agar (commercial)	37	80	1	
		270	1½	
Gracilaria confervoides Extract (South Africa)	37	187	1	
		337	1½	
Japanese agar	38	70	1	
		80	1½	
Reclaimed agar	39	113	1	
		287	1½	
Bacto-agar (new sample)	39	215	1	
		350	1½	
Gelidium agar (Lab. extract)	39	360	1	
		450	1½	
Gracilaria confervoides extract (North Carolina)	43 and 59	113	½	
		407	1	
		650	1½	
Gracilaria cornea (extract) (Florida)	46	0	1	
		10	2	
		15	3	Solution viscous
Gracilaria confervoides (Australia) extract	46	95	1	
		167	1½	
Gracilaria confervoides (California) extract	47	120	1	
		190	1½	
Gracilaria floridana (Florida) extract	48	20	1	
		110	2	Solution viscous
		210	3	

* Grams per centimeter plunger circumference.

To obtain data to be used as a basis for suggesting standards of identity and quality, analyses were made of 73 randomly selected samples from the government stockpile. These consisted entirely of Japanese agar. These data show that the protein content varied from 1.3 to 4.2 percent, with 86 percent of the samples containing less than 1.8 percent. The water-insoluble debris varied from 0.3 to 1.1 percent except for a few dirty samples which contained upwards to 7.8 percent of

this material. The ash content varied from 3.0 to 7.0 percent. Most of the samples contained about 4.0 percent ash. At least 70 percent of the samples contained from 50 to 90 milligrams of iron and from 4 to 8 milligrams of copper per kilogram of agar, plus not more than 0.25 percent of acid-insoluble ash.

No sample gelled at 45° C., and all gelled at 35° C., under the conditions of the test. A range in viscosity from 3.5 to 8.0 centipoises at 45° C., included 88 percent of the samples. The breaking load showed a small frequency peak at 80 and a high peak at 120 grams per centimeter of plunger circumference. The syneresis amounted to 0.5 to 1.0 milliliters of free liquid per 100 grams of gel. These physical characteristics were determined using gels of 1.5 percent (dry weight) concentration.

In comparison, gels of 1.5 percent (dry weight) concentration prepared from 50 samples of laboratory-extracted gum of Gracilaria confervoides from North Carolina showed breaking loads varying from 60 to 340 grams per centimeter of plunger circumference. The syneresis ranged from 0.9 to 5.0 milliliters of free liquid per 100 grams of gel. About 80 percent of the samples gelled at a temperature higher than 45° C. The ash content of four samples from several sources, including a purified commercial sample, ranged from 5.5 to 8.0 percent.

These samples of Gracilaria gum were found unsuitable as a substitute for agar in bacteriological media because of excessive syneresis, high viscosity, and non-uniform gelation. The latter characteristic was demonstrated by the presence of lumps of gel in solutions held at temperatures above that permitting complete gelation. These lumps caused difficulty in pouring plates.

The Service will publish more extensive analyses of data obtained in experimental work on this subject, and an inquiry should be made for available publications in the event that this summary does not suffice.